

The University of Chicago

EFFECTS OF FLUORESCEIN AND
PHOTOSENSIN ON GROWTH
OF RED KIDNEY BEANS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1943

By
ROBERT K. ZUCK

Reprinted from
THE BOTANICAL GAZETTE
Vol. 106, No. 1, September, 1944

The University of Chicago

EFFECTS OF FLUORESCEIN AND
PHOTOSENSIN ON GROWTH
OF RED KIDNEY BEANS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1943

By
ROBERT K. ZUCK



Reprinted from
THE BOTANICAL GAZETTE
Vol. 106, No. 1, September, 1944

EFFECTS OF FLUORESCIN AND PHOTOSENSIN ON GROWTH OF RED KIDNEY BEANS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 563

ROBERT K. ZUCK

Introduction

Many organic compounds are known to have local growth-regulating effects on plants. Attempts to disclose general stimulation of growth by these compounds have been undertaken many times, but critical work has revealed little or no stimulation of the growth of the entire plant by any of the compounds examined (2, 8). Among those tested were indoleacetic acid, indolebutyric acid, naphthaleneacetic acid, naphthalene acetamide, and phenylacetic acid. At the present time there is apparently no experimental evidence indicating stimulation of growth of the entire plant by this class of growth-regulating substances.

Recently it has been stated (6, 7) that fluorescein acts as an inhibitor and as a stimulator of plant growth, depending upon its concentration. This compound is not known to have local growth-regulating effects. In their work with fluorescein, SELLEI, SELLEI, MAYER, and WENT (7) have employed a commercial preparation (Photosensin) composed of 93.9% sodium bicarbonate, 5% fluorescein, 1% iron sulphate, and 0.1% copper sulphate. Where inhibition or stimulation of plant growth has resulted from the application of varying amounts of Photosensin, the effects on the plant have been attributed to the fluorescein content and not to any one or all of the other three compounds of the preparation. In the one case where Photosensin was not used, fluorescein was presumably employed with sodium bicarbonate to keep the high concentrations of fluorescein in solution. In addition, the experiments were carried out on a small

number of plants for each treatment where a synthetic substratum was used. Five plants per treatment are not sufficient to overcome individual variation in the case of such variable species as marigold and tomato when grown from seed.

The experiments reported here were designed to investigate further the effects of fluorescein, of Photosensin, and of the inorganic constituents of Photosensin on more extensive populations of a single variety of plant grown on a synthetic substratum.

Material and methods

Red kidney beans were grown under greenhouse conditions at two separate times, from October 9 to November 6, 1942; and from May 25 to June 14, 1943. In both cases growth was terminated when flower buds began to appear. The lot of seeds employed in the 1942 experiment proved irregular in germination, emergence, and development. The epicotyls of many seedlings did not elongate or continue development, although some plants produced shoots from buds in the axils of the cotyledons. Only those plants in which the epicotyl continued to develop were used, however, those with shoots produced from buds in the axils of cotyledons being discarded. A second lot of seed employed for the experiment of 1943 gave a very uniform stand of vigorous plants, of which the first simple leaves were exceptionally large.

Two plants were grown in 5 lb. each of pure quartz sand in glazed jars with side drainage at the bottom, the hole being covered with glass wool. Seeds

were planted four to a jar at 1-inch depth and immediately supplied with complete nutrient, the sand previously having been rinsed twice with distilled water. Just after the hypocotyl arch had straightened out, the seedlings were thinned to two per jar.

The nutrient medium consisted of 0.0060 M calcium nitrate, 0.0045 M magnesium sulphate, and 0.0045 M potassium-acid phosphate in distilled water. Microelements were supplied as 0.1 p.p.m. iron sulphate, 1.0 p.p.m. zinc chloride, 1.0 p.p.m. manganese sulphate, and 1.0 p.p.m. sodium tetraborate. No copper was added to the first series but was added as 1:50,000,000 copper sulphate in the second experiment to keep the medium comparable with that used by SELLEI *et al.* (7). All chemicals were of c.p. grade. The fluorescein was obtained from Eastman Company and the Photosensin was the commercial product of that name.

Hydrogen and hydroxylion determinations were made on the Coleman glass electrode electrometer no. 3. Osmotic pressures were determined according to the freezing-point depression method of LOOMIS and SHULL (4).

Harvesting was by rows of seven jars each, the tops being cut off at sand level and the roots rinsed free of sand in tap water. Both tops and roots were dried in a hot-air oven at 60°–70° C. for 18 hours. The dried samples were then placed in a desiccator over calcium chloride until constant weights were obtained. Weights were taken to the nearest hundredth of a gram.

The first experiment consisted of two treatments and a control. The concentrations of fluorescein were 1:50,000 and 1:500,000, the control being plain nutrient. There was some precipitation of the fluorescein at the 1:50,000 concen-

tration, but this was stirred up each time the solution was applied. The pH of the nutrient was 4.0 and was not affected by the addition of the fluorescein. Each treatment comprised 140 plants arranged in ten rows of seven jars each, the resulting thirty rows randomized on one bench in the greenhouse. Randomization was accomplished by assigning numbers to the treatments, shaking them in a container, and drawing them off until each row had been assigned the type of treatment. The plants were thinned to two per jar and treatment begun on October 15, 1942, 6 days after the seeds were placed in the sand. Solutions of the corresponding type were applied when the surface of the sand began to appear dry. This amounted to five applications of about 250 ml. each. The plants were harvested on November 6, 1942.

The five treatments (and a control) of the second experiment, the plants randomized as in the first experiment, were designed to observe the effect of a concentration of fluorescein of 1:10,000,000 and the accompanying amounts of inorganic compounds found in the quantity of Photosensin required to obtain this concentration of fluorescein. Each treatment and control consisted of 140 plants, following the method of the first experiment. A concentration of fluorescein of 1:10,000,000 was chosen, since the data of SELLEI (7) indicate this to be near the optimum. The treatment consisted of the following concentrations in nutrient solution: fluorescein 1:10,000,000; sodium bicarbonate 18.8:10,000,000; iron sulphate 0.2:10,000,000; copper sulphate 0.02:10,000,000; Photosensin to make 1:10,000,000 fluorescein.

The pH of the five dilute solutions of the second experiment was taken before and after addition of the substances. No

changes were observed. Four of the solutions were pH 4.0, the one with sodium bicarbonate being pH 3.95. Thinning of the plants and application of the treatments was done in May, 1943. The longer growing period required seven applications of about 250 ml. Harvesting occupied 3 days. All tops were cut on June 14, 1943. The roots were harvested on the two succeeding days, one bench on each day.

A third experiment was run on a smaller scale to observe the effects of considerable amounts of sodium bicarbonate over a range of concentrations with and without fluorescein, as this compound is the largest (93.9%) constituent of Photosensin. The sodium bicarbonate and fluorescein concentrations per liter of nutrient, together with the pH, were as follows:

Sodium bicarbonate		fluorescein, pH
56.4 gm., without		pH 8.40
56.4	with 3 gm.	pH 7.90
18.8	without	pH 8.40
18.8	with 1 gm.	pH 7.95
9.4	without	pH 8.30
9.4	with 0.5 gm.	pH 8.20
4.7	without	pH 8.05
4.7	with 0.25 gm.	pH 8.75

The solutions were applied to two jars of two to four plants for each treatment. The osmotic pressure of the solution containing 56.4 gm. of sodium bicarbonate per liter of nutrient was 24.85 atmospheres; that of the same amount of sodium bicarbonate plus 3 gm. of fluorescein was 21.39 atmospheres. No weights were taken on the plants of this experiment, since they were killed by the three highest concentrations of sodium bicarbonate and of it plus fluorescein. One application of each treatment was sufficient to produce the effects noted.

Results

In the first experiment employing only fluorescein, no consistent differences were noticeable among the living plants. Some irregularities occurred, but these could not be related to any treatment. Table 1 lists the dry weights of roots and tops, each weight representing the tops or roots of fourteen plants, to-

TABLE 1

FLUORESCEN IN RELATION TO GROWTH OF
RED KIDNEY BEANS. DATA IN GRAMS
DRY WEIGHT OF FOURTEEN PLANTS

CONTROL (PLAIN NUTRIENT)		FLUORESCEN IN NUTRIENT SOLUTION			
		1:50,000		1:500,000	
		Tops	Roots	Tops	Roots
12.54	3.90	12.66	1.04	13.54	2.30
14.60	3.12	14.68	1.97	18.87	2.84
13.56	3.04	12.20	1.55	12.31	3.14
14.13	3.51	15.48	3.80	9.31	2.83
11.91	2.95	16.16	5.94	14.25	5.94
11.22	3.67	14.91	3.84	12.67	5.02
12.36	2.50	11.89	5.43	12.53	4.72
13.91	3.57	13.39	5.35	10.57	5.70
13.06	4.57	14.58	4.60	9.01	2.52
16.94	4.94	11.94	4.97	12.15	4.44
134.23	35.57	137.89	38.49	118.21	39.45
Total .	169.80	Total .	176.38	Total .	157.66

gether with the total weights of tops and roots for each treatment. The weight of the plants treated with the 1:500,000 dilution is 12.14 gm. less than the control, and the plants treated with the 1:50,000 dilution weigh 6.58 gm. more than the control. On a percentage basis, the plants of 1:50,000 dilution weigh 3.8% greater, and those treated with the 1:500,000 dilution weigh 6.2% less, than the controls.

In comparing the data in tables 1 and 2, greater regularity in weights are shown in table 2, indicating an even

more uniform stand of plants. Likewise, no visible differences were observed in the growing plants of the second experiment. The copper-sulphate treated plants of the second experiment weighed the most, with the fluorescein- and Photosensin-treated plants second and third highest in weight, respectively. The plants of the control and those treated with iron sulphate and sodium bicar-

were killed. All plants receiving fluorescein plus sodium bicarbonate and sodium bicarbonate alone in the three highest concentrations were dead 1 day after treatment. Those receiving the lowest concentrations showed drying at the edges of the oldest leaves at the end of 2 days, when the experiment was terminated. The plants were of the same stage of development as were those of the first

TABLE 2

GROWTH OF RED KIDNEY BEANS IN RELATION TO FLUORESCEIN, PHOTOSENSIN, AND INORGANIC CONSTITUENTS OF PHOTOSENSIN. CONCENTRATIONS AS INDICATED IN NUTRIENT SOLUTION. DATA IN GRAMS DRY WEIGHT OF FOURTEEN PLANTS

CONTROL (PLAIN NUTRIENT)		0.2:10,000,000 IRON SULPHATE		1:10,000,000 FLUORESCEIN		18.8:10,000,000 SODIUM BICAR- BONATE		0.02:10,000,000 COPPER SULPHATE		PHOTOSENSIN TO MAKE 1:10,000,000 PHOTOSENSIN	
Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
20.61	5.13	21.28	4.17	22.07	4.60	21.93	4.74	23.87	5.35	20.47	4.95
23.31	7.07	23.20	6.19	22.29	5.20	22.59	4.65	23.30	4.51	23.73	6.03
22.96	5.28	23.38	5.63	23.23	4.71	20.27	6.78	23.65	5.14	23.14	4.68
18.47	4.95	21.56	4.45	21.73	4.64	21.33	4.44	23.43	5.88	23.75	5.38
24.29	5.15	23.26	4.85	21.93	4.35	20.90	5.02	24.10	5.14	21.82	4.12
20.75	5.19	22.04	4.56	22.60	4.40	22.54	4.54	22.71	4.81	22.14	4.16
20.95	4.56	21.99	4.63	25.19	5.95	23.77	4.75	22.48	4.29	21.54	4.79
22.98	4.95	22.49	4.26	23.33	4.55	21.50	4.04	23.73	5.65	22.30	4.93
21.51	4.46	21.81	4.81	22.51	4.47	22.74	4.64	21.12	4.19	21.97	5.00
21.21	5.17	20.95	4.82	22.20	5.28	22.55	5.00	22.18	5.17	23.15	5.72
217.04	51.91	221.96	48.37	227.08	48.15	220.12	48.60	230.57	50.13	224.01	49.76
Total..	268.95	Total..	270.33	Total..	275.23	Total..	268.72	Total..	280.70	Total..	273.77

bonate were all very nearly the same weight.

The sodium-bicarbonate treatments of the third experiment with and without fluorescein in the three highest concentrations killed the plants. The highest concentration of sodium bicarbonate alone, and with the 3 gm. of fluorescein, produced noticeable effects on the plants within 5 minutes after application. At the end of 15 minutes the plants had collapsed. Fluorescein began to show in the veins within half an hour after treatment, indicating that the root systems

and second experiments when treatments began; that is, the hypocotyl arch had just straightened out.

Discussion

In considering the data presented by SELLEI *et al.* (7) for the sand culture experiments consisting of five plants per treatment, a lack of consistency over the range of treatments is apparent. Although most of the treated plants developed higher weights than the controls, these in several instances are higher than the treated plants. Furthermore,

the series of treatments do not form a smooth curve of increasing weights with decreasing concentrations, or vice versa. Nor can the effect on the plants logically be attributed to the fluorescein in the Photosensin preparation employed by them. As was pointed out earlier in this paper, Photosensin is composed of four compounds, three inorganic ones and the organic fluorescein. The inorganic compounds have cations definitely known to have effects on the growth of plants, the anions not being important in the concentrations used. Likewise, the stunting effect attributed to the fluorescein in high concentrations cannot be divorced from the inorganic compounds of the Photosensin preparation.

The lack of pronounced visible differences among the living plants and the small differences in dry weights shown in tables 1 and 2 indicate that fluorescein by itself in the three concentrations employed is without appreciable effect on the growth of red kidney beans. If the slight additional weight made by the plants treated with the 1:50,000 fluorescein concentration was attributable to the dye, then the slightly less weight made by the plants receiving 1:500,000 and 1:10,000,000 fluorescein cannot easily be explained as an actual inhibition of growth. Rather, the small differences in dry weights and appearance of the living plants indicate that these are within the variations of the populations of plants employed.

The data supplied by the second experiment do not appear to show significant differences, inasmuch as the greatest weight was made by those plants receiving the slight additional copper found at a concentration of fluorescein of 1:10,000,000 as Photosensin. In all probability, the variations in weights are

those to be expected with populations of this size under ordinary conditions.

Sodium, which is in dispute as an essential element for plants, is not without effect on plants and cannot be ignored when used in low concentrations as sodium bicarbonate in the Photosensin preparation. MULLISON and MULLISON (5), working with barley, found evidence of substitution for potassium at almost all levels of potassium and sodium.

Any further experimentation to observe the effects of fluorescein as a stimulator of plant growth must take into account that Photosensin is a preparation consisting of fluorescein, sodium bicarbonate, iron sulphate, and copper sulphate. If the effects are to be referred to fluorescein, this dye must be employed by itself.

Those plants receiving the high concentration of sodium bicarbonate and sodium bicarbonate-fluorescein in nutrient solution were killed by the treatments. The effects on the plants cannot be called merely stunting of growth, for death ensued within a matter of minutes after the plants had been subjected to the treatments. The killing is about as rapid when the sodium bicarbonate-fluorescein solutions are used as when the sodium bicarbonate alone is applied. Although the pH values are relatively alkaline for good growth, the rapidity with which these plants were killed points rather to the high osmotic pressures (24.85 atmospheres for the highest concentration of sodium bicarbonate) to which the roots were subjected. The precipitation of much of the magnesium and calcium in the form of carbonates by the extensive amounts of sodium bicarbonate removes the buffering qualities of these metallic ions from the solu-

tion. Likewise, the nitrate and sulphate ions released by these precipitated cations allow for more thorough ionization of the sodium in solution. All these factors probably contribute to the rapidity with which the plants are killed, but it would appear that the high osmotic pressures are largely responsible.

HAYWARD and LONG (3) and GERNER (1), working with high osmotic pressures attained by sodium sulphate and sodium chloride in nutrient solution, found that killing was common in the higher concentrations of these salts. HAYWARD and LONG report that killing was complete for tomato at an osmotic pressure of 6 atmospheres attained by the addition of sodium sulphate to the nutrient solution, whereas GERNER reports killing as very frequent for tomato at the highest concentrations, between 11 and 12 atmospheres. In both papers it is stated that the high osmotic values were attained by gradually increasing the amounts of solutes over a period of days. HAYWARD and LONG do not report a sodium toxicity as such, but they do note that "it seems possible, however, that the sodium ion may have an inhibitory effect on growth."

Thus, it can be assumed that the rapid change in the physico-chemical envi-

ronment of the root systems of the plants subjected to the extensive amounts of sodium bicarbonate caused death of the plants, and the observed effects cannot be attributed to the fluorescein when it is kept in solution by sodium bicarbonate.

Summary

1. Red kidney beans were grown in pure quartz sand with nutrient solution to which were added in separate series Photosensin and the constituents of Photosensin—fluorescein, iron sulphate, sodium bicarbonate, and copper sulphate.

2. There were no pronounced visible differences among the living plants, and the differences in dry weight were small. Those plants receiving the small amount of copper sulphate weighed the most.

3. Apparently fluorescein does not stimulate growth of red kidney beans.

4. The so-called stunting effect heretofore ascribed to the high concentrations of fluorescein is shown to be actual killing by the extensive amounts of sodium bicarbonate used to keep the fluorescein in solution.

BUREAU OF PLANT INDUSTRY, SOILS
AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

LITERATURE CITED

1. GERNER, GLADYS, Effects of chlorides on chlorophyll content of tomato. Unpublished Thesis, Univ. of Chicago. 1939.
2. HAMNER, M. E., Effects of phenylacetic acid and naphthalene acetamide on tomato plants grown in soil. *BOT. GAZ.* 103:576-580. 1942.
3. HAYWARD, H. E., and LONG, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride and sodium sulphate and nutrient solutions. *BOT. GAZ.* 102:437-462. 1941.
4. LOOMIS, W. E., and SHULL, C. A., Methods in plant physiology. New York. 1937.
5. MULLISON, W. R., and MULLISON, ETHEL, Growth responses of barley seedlings in relation to potassium and sodium nutrition. *Plant Physiol.* 17:632-644. 1942.
6. SELLEI, J., Further growth experiments with fluorescent dyes. *Growth* 5:27-52. 1941.
7. SELLEI, J., SELLEI, H., MAYER, A., and WENT, F. W., Effects of fluorescein on plant growth. *Amer. Jour. Bot.* 29:513-522. 1942.
8. STEWART, W. S., and HAMNER, C. L., Treatment of seeds with synthetic growth-regulating substances. *BOT. GAZ.* 104:338-347. 1942.

